mutations to generate probes capable of differentiating action potentials from sub-threshold synaptic activity, and hyper-polarizing inhibitory activity.

755-Pos Board B535

Transition Metal Ion FRET Measurements in Plasma Membrane Sheets Prepared by Deroofing Cultured Mammalian Cells

Eric Senning, William N. Zagotta, Sharona E. Gordon.
Department of Physiology and Biophysics, University of Washington, Seattle, WA, USA.

Since the discovery of Förster resonance energy transfer (FRET) in the 1940s, its implementation to measure inter- and intramolecular distances has reached wide-spread use in the biological sciences. Because the technique requires labeling of a donor and acceptor site with two different spectroscopically active molecules, a prominent difficulty with biological samples has been how to label with specificity and, therefore, unambigouisly detect and assign the signal contribution from each site. Here we show that transition metal ion FRET (tmFRET) experiments using plasma membrane sheets prepared from deroofed cells can be used as an orthogonal labeling scheme with an unambiguous assignment of the FRET pair signal. As a FRET donor, we used octadecyl rhodamine B (R18), which incorporated into the plasma membrane of deroofed cultured cells. To measure distances on the length scale relevant to membrane proteins, we used the transition metal ion Co^{2+} as an acceptor, which, together with R18, gives a R_0 value of 14.9 Å. Although the quenching by Co^{2+} of rhodamine B in solution is of low affinity (>10 mM), 100 μM Co^{2+} caused appreciable quenching of R18 incorporated into plasma membrane sheets. By first introducing a synthetic fatty acid with a metal-chelating head group, appreciable quenching was observed with less than 1 μM Co^{2+}. Comparison of tmFRET experimental measurements to expected theoretical values convincingly demonstrate that these experiments provide information about the distance of closest approach between donor and acceptor (Fung and Stryer, 1978). These experiments provide proof-of-priniciple for measurements of biologically relevant distances in native membranes. We anticipate that FRET experiments on plasma membrane sheets will offer a unique opportunity to explore protein-lipid interactions and discuss several possible future directions for this technique.

756-Pos Board B536

Development of Novel FRET-Based Fluorescent Voltage Sensor Proteins

Masoud Sepehri Rad[1], Uhna Sung[1], Thomas Hughes[2], Lawrence B. Cohen[3], Bradley J. Baker[1].
[1]1Center for Functional Connectomics, Korea Institute of Science & Technology, Seoul, Korea, seoul, Korea, Republic of, [2]Department of Cell Biology and Neuroscience, Montana State University, Bozeman, MT, USA, bozeman, MT, USA, [3]Center for Functional Connectomics, Korea Institute of Science & Technology, Seoul, Korea, seoul, Korea, Republic of.

Recently, we developed a series of FRET (Fluorescence Resonance Energy Transfer)basedvoltage sensors, Nabi,utilizing the voltage sensing domain of CiVSP (*Ciona intestinalis* voltage sensitive phosphatase). The Nabi series of probes contained FPs inserted at different locations in the *Ciona* voltage sensitive domain.Nabi2 probes with Clover (green) and mRuby2 (red) as donor and acceptor showed large signal size (9% ΔF/F for a 100 mv depolarization) and fast taus (<3 msec). In order to improve further FRET efficiency of voltage responses, we investigated the behaviors of individual FPs in the probes. When we deleted the acceptor FP at the C-terminus of Nabi2, the signal size (ΔF/F) was reduced from 9% to 1 %. The optical responses of these probes does not appear to arise from the signals of individual FPs. The FRET interactions of 2 FPs are important for the signals of the Clover/mRuby pair. For further investigation, we also would like to remove the donor. We also plan to test additional FPs in the Nabi series. We hope that this effort will result in better FRET based voltage probes.

Supported by US NIH Grants DC005259 and NS054270 and grant WCI 2009-003 from the National Research Foundation of Korea.

757-Pos Board B537

Improving Signal Dynamics of Fluorescent Protein Voltage Sensors by Optimizing FRET Interactions

Uhna Sung[1], Lei Jin[2], Thomas Hughes[3], Lawrence B. Cohen[1], Bradley J. Baker[1], Masoud Sepehri-Rad[1].
[1]CFC, KIST, Seoul, Korea, Republic of, [2]Molecular and Cellular Physiology, Yale Universiy, New Haven, CT, USA, [3]Cell Biology and Neuroscience, Montana State University, Bozeman, MT, USA.

FRET (Förster Resonance Energy Transfer)-based voltage sensors can be useful for monitoring neuronal activity *in vivo* because the ratio of signals between the donor and acceptor pair removes common sources of noise such as heart beat artifacts. We improved the performance of genetically encoded fluorescent voltage sensors by optimizing FRET interactions between donor and acceptor fluorescent proteins (FPs) flanking the voltage sensitive domain of the *Ciona intestinalis* voltage sensitive phosphatase. The donor FP, UKG, was positioned at 8 different locations downstream of the voltage-sensing domain while the acceptor FP, mKO, was placed at 6 positions upstream of the voltage-sensing domain resulting in 39 different "Nabi1" constructs. Several of these combinations resulted in large signals and fast kinetics. Nabi1 probes responded to changes in membrane potential with improved signal size (up to 11% ΔF/F for a 100 mv depolarization) and fast response time constants both for signal activation (~2 ms) and signal decay (~3 ms). Replacement of the mKO and UKG FRET pair with Clover (donor FP) and mRuby2 (acceptor FP) improved expression in cultured neurons enabling the optical resolution of individual action potentials. We are analyzing a series of Nabi2 probes with different insertion sites of FPs. This effort may identify novel probes that may be useful for optical recording of neuronal activities.

Supported by US NIH Grants DC005259 and NS054270 and grant WCI 2009-003 from the National Research Foundation of Korea.

758-Pos Board B538

Electrophysiological Characterization of Purkinje Cells from FHM3 (SCN1A) Knock-In Mice

Ilaria Zanardi[1], Sara Bertelli[1], Nikolaus Plesnila[2], Tobias Freilinger[3], Paola Gavazzo[1], Michael Pusch[1].
[1]IBF CNR, Genova, Italy, [2]Institute for Stroke and Dementia Research, University of Munich Medical School, Munich, Germany, [3]Department of Neurology and Epileptology, University of Tuebingen, Tuebingen, Germany.

Migraine is a particularly severe form of headache which arises in only one side of the head and affects women (13-20%) more often than men (3-14%). Familial hemiplegic migraine type 3 (FHM3) is a rare autosomal dominant genetic disease, whose major symptoms are severe migraine, hemiparesis and aura. FHM3 is caused by mutations in the gene coding for the voltage-dependent sodium channel Nav1.1, whose activity is responsible for the generation and transmission of the fast electrical signals used by neurons to communicate across long distances. The specific molecular mechanisms that underlie the migraine phenotype of some SCN1A mutations are not fully understood, since conflicting results have been reported for the properties of several FHM3-mutations in heterologous expression systems (Vanmolkot et al, 2007, Hum Mut 28:522; Kahlig et al, 2008, PNAS 104:9799; Cestele et al, 2013, PNAS, 110:17546). Taking advantage of a novel mouse knock-in model carrying the L1649Q mutation, the electrophysiological properties of cerebellar Purkinje cells have been investigated from WT, heterozygous and homozygous knock-in mice in terms of activation, inactivation, slow inactivation, and persistent Na(+) current which will help to understand the impact of the mutation in the neuronal network. The biophysical characterization of the mutants are also compared with those seen in the context of the SCNA5 channel (Vanmolkot et al, 2007) and with those seen in HEK293 cells (Cestele et al, 2013), where, the mutation causes slower inactivation and other features consistent with a gain of function phenotype.

759-Pos Board B539

Bidirectional Propagation of Action Potential in Giant Axons of Nerve Bundles from Homarus Americanus

Tian Wang, Alfredo Gonzalez-Perez, Jorin Diemer, Søren Nissen, Thomas Heimburg.
Niels Bohr Institute, Copenhagen, Denmark.

For decades, it was widely accepted that the propagation of action potential in neurons is unidirectional, down the axon. Since the 1950s, evidence has shown that an action potential can also propagate back through the dendrites sending a retrograde signal to its presynaptic signaling neurons. The backpropagation of action potentials has been extensively investigated in dendrites ever since but less attention has been paid to signal propagation in axons due to widely accepted passive role of axons in signal propagation.

In our experiments nerve bundles from the walking legs of lobster, *Homarus americanus* were used, to investigate the bidirectional propagation of action potentials in axons. Stimulation in ectopic sites on the giant axons results in bidirectional propagation of action potentials. Our results show an asymmetrical propagation of the orthodromical and antidromical signals. The conduction velocity of the signals propagating in both directions were correlated with the stimulation voltage, staying constant or decreasing as a function of the stimulation voltage depending on propagation direction in correlation with the difference in axonal diameter. We interpret our results with the soliton model that assumes a gel phase solitary wave propagating across a fluid phase along the lipid membrane in the axon. The results are in good agreement with the theoretical predictions for the conduction velocity dependence on energy for soliton waves.